

Steven M. Ruben
Appl. No. 10/662,429

Department _____

Subject _____

Name GUO-Liang YU

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Computation Notebook

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Ruben EXHIBIT 2063
Ruben v. Wiley et al.
Interference No. 105,077
RX 2063

1/23/96 Large scale plasmid prep for cDNA library

HBC to be used to make single stranded DNA

to test SAGE

— transformation from an old stock of plasmid DNA

— obtain titer 1.5×10^7 cfu grow up to 1 L O/W

— plasmid prep

spin down, resuspend in 40 μ l H₂O
add 80 μ l NaOH / SDS
60 μ l 3M KOAC

spin down ppt with isopropanol

CsCl₂ gradient 60K g/h

Concentration of DNA is low

2

1/24 proliferation assay for endothelial cells

EDAP

cell lines Artery endothelial cell
Vein endothelial cells
HUVEC

Seed 5×10^3 cells to each 96 well DMEM 10% FBS
5 hr incubation — let cell attach, remove medium
add samples as show below

	TNF α				EDAP E2				EDAP E3			
	1	2	3	4	5	6	7	8	9	10	11	12
A	/	1 ng			1 μ s				1 μ s		10%	✓
B	/	100 ng			100 ng				100 ng		1%	✓
C	/	10 ng			10 ng				10 ng		0.1%	✓
D	/	1 ng			1 ng				1 ng		0.01%	✓
E	/	bfr			1 μ s				100 ng		10%	✓
F	/	bfr			100 ng				10 ng		1%	✓
G	/	Control			10 ng				1 ng		0.1%	✓
H	/	Control			1 ng				0.1 ng		0.01%	✓

EDAP
E4

bfr

40 hr after TNF α assay for A β & VC
add 3H thymidine
incubate 6 hrs
wash once w/ PBS. Lyse cell Count

Certificate of Analysis

3

Protein Name: TNF gamma (EDAP)

Batch #: HG02704-E2

Date: 11/10/95

Expression System: E. Coli

Method of Purification:

1. Cell disruption and purification of inclusion body
Wash pellet with TE, 1M, 2 M Urea, and
2. Extraction TNF γ with 4 and 8 M Urea
3. Buffer exchange (2XPBS) and refolding in PD-10
4. Removing endotoxin by affinity gel chromatography

Estimated Purity: > 80%



TNF gamma

Protein Concentration:

Method A: 176 μ g/ml

Method B: 180 μ g/ml

Endotoxin level in sample: 0.17 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Sterile filtered: Yes ☒ No ☐

Buffer composition: 2XPBS

Total amount of protein purified: 14 mg

Prepared by: Jian Ni

Approved by: *Kevin Jeff*

1.5nd 5/3 11/30

Updated May 23, 1995

Certificate of Analysis

Protein Name: TNF gamma

Batch #: HG02703-3E

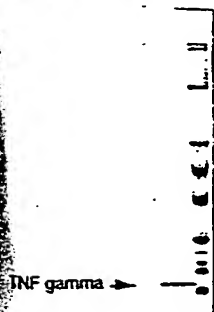
#3 5/5 ~ 5/66 Date: 5/26

Expression System: E. Coli

Method of Purification:

1. Cell disruption and purification of inclusion body
2. Solubilization of protein with 8 M urea
3. Sephadex S-200 size exclusion chromatography
4. Dialysis into PBS

Estimated Purity: > 70%



TNF gamma

Protein Concentration: 80 μ g/ml

Endotoxin level in sample: 525 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Buffer composition: PBS

Endotoxin level in buffer: <10 EU/ml

Sterile filtered: Yes ☒ No ☐

Prepared by: Jian Ni

Approved by: *Kevin Jeff*

Updated May 23, 1995

Certificate of Analysis

Protein Name: TNF-delta (His-tag)
Clone ID#: HLTBT71

Batch #: HG10700-E1

Date: 1/22/96

Expression System: E. Coli

Molecular weight: 20.8 Kd

Method of Purification:
Nickel-chelate affinity chromatography column
Buffer exchange (2XPBS) and refolding in PD-10 column

Estimated Purity: 80%



Protein Concentration:

Method A: 200 µg/ml

Method B: 160 µg/ml

Endotoxin level in sample: 2.8 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Sterile filtered: Yes Y No

Buffer composition: 2XPBS

Biological activity
yes/not tested

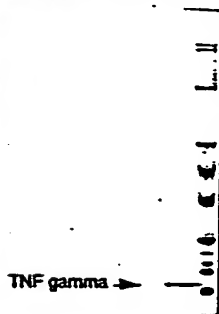
Total amount of protein purified: 10 mg

Prepared by: Jian Ni

Approved by:

Updated September 15, 1995
4, Dialysis into PBS

Estimated Purity: > 70%



Protein Concentration: 80 µg/ml

Endotoxin level in sample: 525 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Buffer composition: PBS

Endotoxin level in buffer: <10 EU/ml

Sterile filtered: Yes XXX No

Prepared by: Jian Ni

Approved by:

Jian Ni

Updated May 21, 1995

Certificate of Analysis

3

In Name: TNF gamma (EDAP)

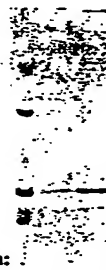
Batch #: HG02704-E2

Date: 11/10/95

Expression System: E. Coli

Method of Purification:
disruption and purification of inclusion body
solubilized with TE, 1M, 2 M Urea, and
refolded TNF gamma with 4 and 8 M Urea
ion exchange (2XPBS) and refolding in PD-10
removing endotoxin by affinity gel chromatography

Estimated Purity: > 80%



TNF gamma

Protein Concentration:

Method A: 176 µg/ml

Method B: 180 µg/ml

Endotoxin level in sample: 0.17 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Certificate of Analysis

Protein Name: TNF-gamma (EDAP) (His-tag)
Clone ID#: HUVEO91

Batch #: HG02702-E4

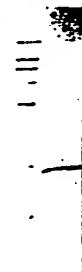
Date: 1/22/96

Expression System: E. Coli

Molecular weight: 20.1 Kd

Method of Purification:
Nickel-chelate affinity chromatography column
Buffer exchange (2XPBS) and refolding in PD-10 column

Estimated Purity: >80%



Protein Concentration:

Method A: 150 µg/ml

Method B: 260 µg/ml

Endotoxin level in sample: 3.1 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Sterile filtered: Yes Y No

Buffer composition: 2XPBS

Biological activity
yes/not tested

Total amount of protein purified: 10 mg

Prepared by: Jian Ni

Approved by:

Updated September 15, 1995

4

MTS assay:

AE

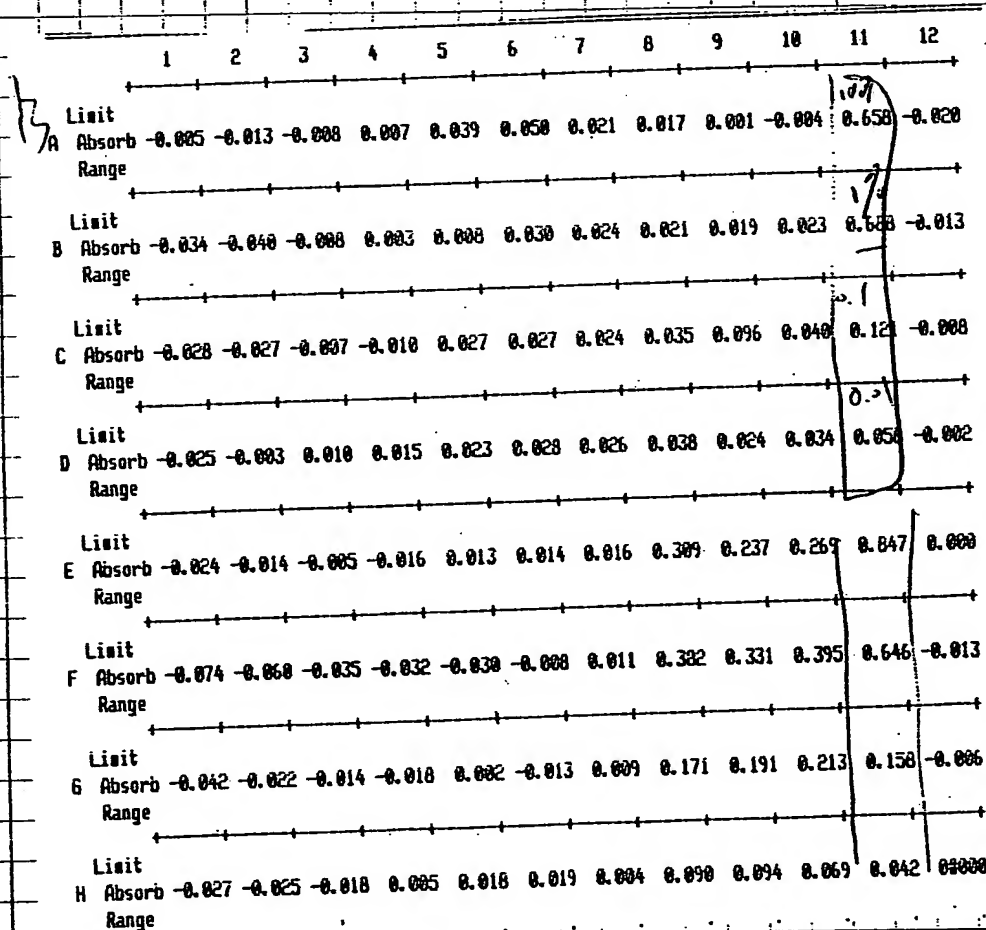
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.019	0.033	0.070	0.033	0.026	0.013	0.006	0	0.012	0.012	0.012	0.012
B	0.47	0.043	0.071	0.058	0.050	0.043	0.047	0.042	0.048	0.048	0.048	0.048
C	0.039	0.071	0.073	0.040	0	0	0.046	0.046	-0.005	0.018	0.018	0.018
D	-0.051	0.011	0.020	0.014	0	0	0	0	-0.04	-0.029	0	0
E	-0.027	0.038	0.043	0.010	-0.029	0.017	0.082	0.077	0.070	0.070	0.070	0.070
F	-0.02	0.066	0.045	0.044	0.033	0.04	0.045	0.033	0.04	0.04	0.04	0.04
G	0.015	0.04	0.028	0.02	0.007	0	0.086	0.091	0.1	0.057	0.057	0.057
H	-0.01	0.061	0.072	0.092	0.039	0.001	0.042	0.013	0.041	0.041	0.041	0.041

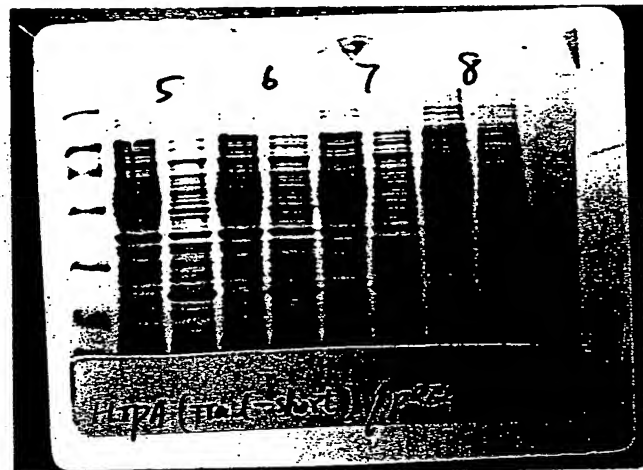
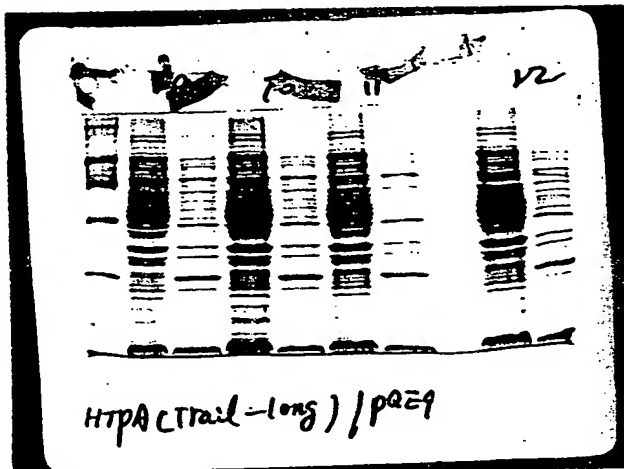
b5

E4

BFGF

FBS



[illegible]

Scanned

3. 4. 2

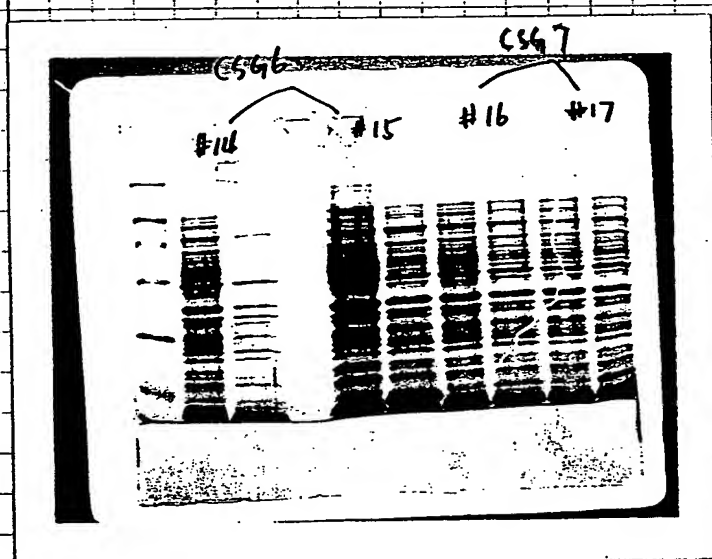
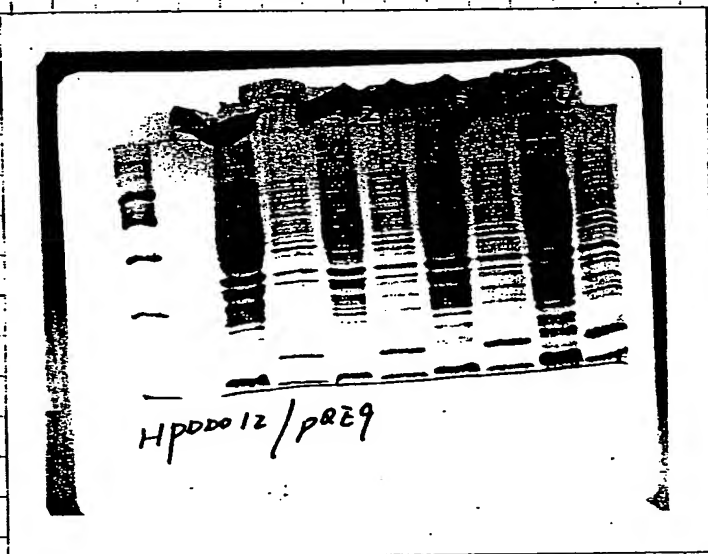
BamHI H-Lys oligos

His tagged

Figure 1. Nucleotide and Predicted Amino Acid Sequence of the TRAIL cDNA

The cDNA sequence of the human TRAIL gene is shown. Numbers at the right indicate the nucleotide number (top) and amino acid number (bottom). Amino acids comprising the predicted transmembrane region are underlined. Underlined nucleotides near the 3' end form a consensus polyadenylation signal.

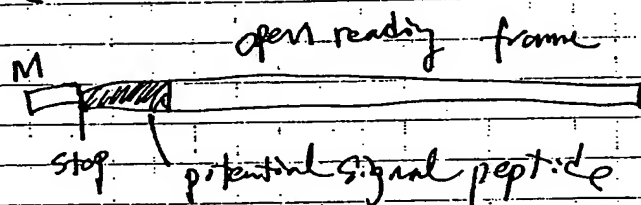
TNF- α expression in pOE9 His tagged



3/12/96 TNF Receptor like HTBN61

Now called Death Domain containing Receptor DDCR

Full length clone HLM4058 contained a stop codon after methionine



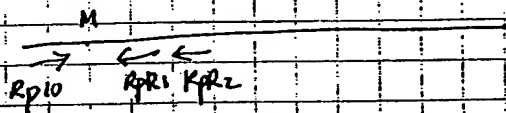
the stop codon is real: with many rounds of sequencing

① to get new clones

② PCR 5' seq direct sequence

oligo HTBN61Rp10 made

CCCTCCGAAACCTGGTG



use Rp10 and RpR2 to PCR libraries. Sequence the product with RpR1

3/13/98 Expression vectors:

- ① EDAP $\Delta 3$ _{d48} 5' CGCCATGGCCTTCACCAAGAACCGA NcoI
② EDAP $\Delta 3$ _{d54} 5' CGCCCATGGACTATACCAASAAATTC NcoI

both clone into pDE60 3' will use EDAP HindIII

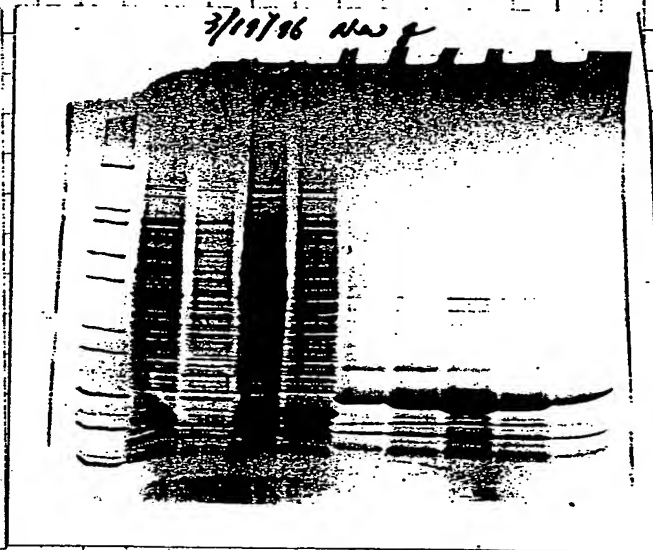
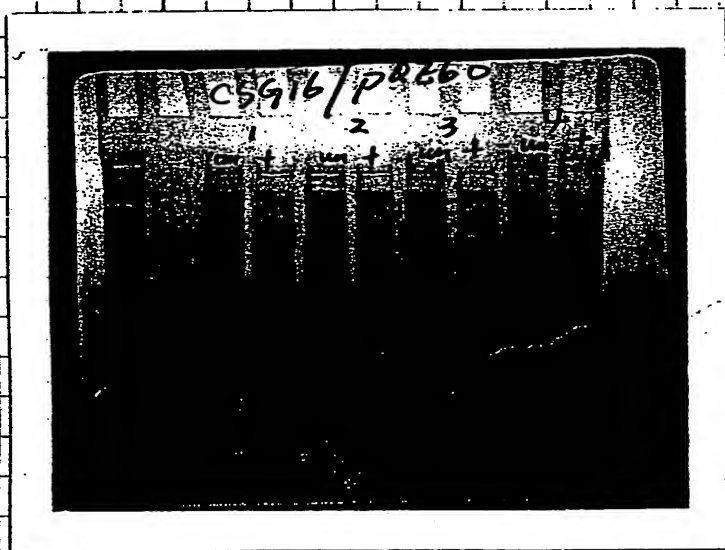
- ③ AIM2 Δ 1 5' CGCCATGGCCAACTCCAGCTTGACC NcoI

- ④ AIM2 Δ 2 5' CGCCATGGTCACCCGCTGCTGCTG NcoI

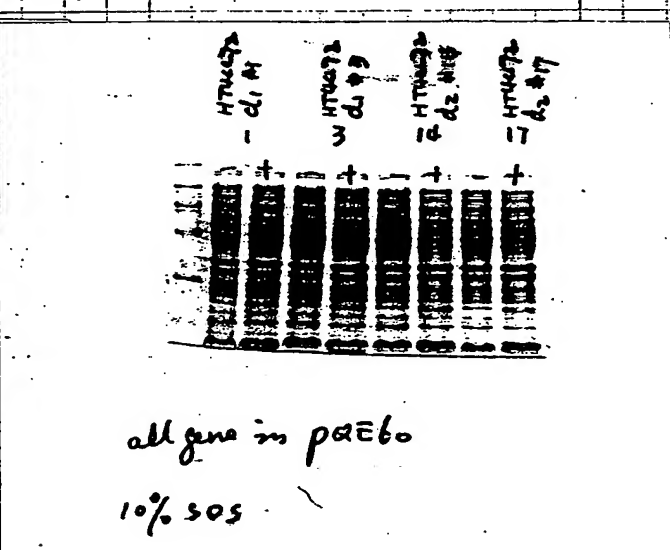
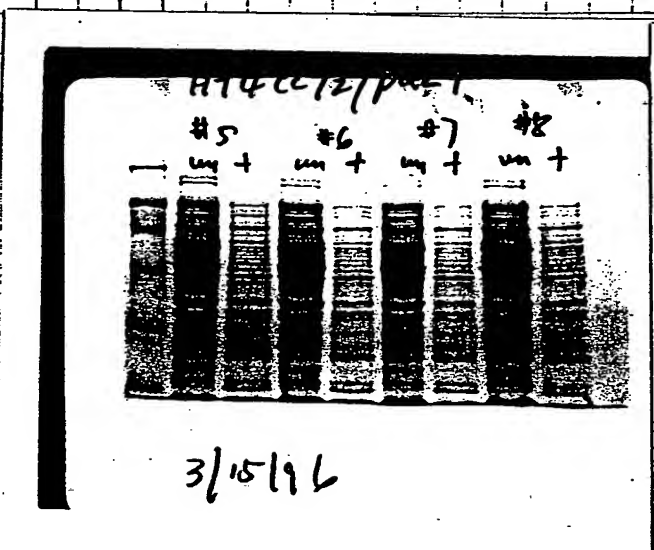
both clone into pDE60 3' HindIII

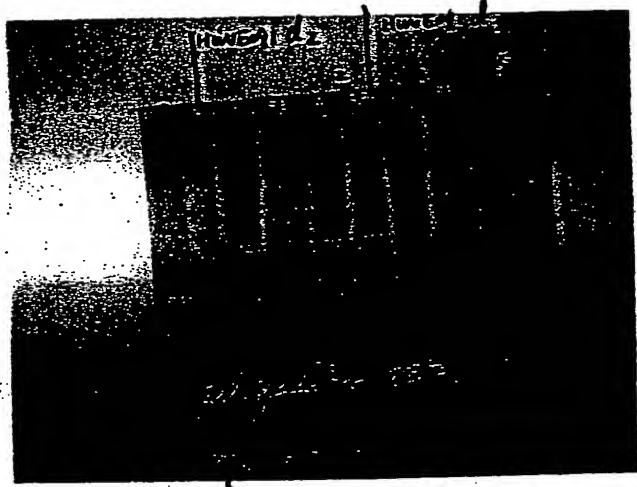
SUPERVISOR
DATE

SUPERVISOR
DATE



Anti-His CS9





EDAP Expression vector position

781 ATATGAGACGCTTTTAAAGCAAAGTCTACAGTTTCCCAATGAGAAAATTAATCCTCTTTC
M R R F L S K V Y S F P M R K L I L F L
PQE9, PQE70, CHO
841 TTGTCTTTCCAGTTGTGAGACAAACTCCACACAGCACTTTAAAAATCAGTTCCAGCTC
V F E V Y R Q T P T Q H F K N Q F P A L
IL6 PQE60, pA2-GP
901 TGCACGTGGGAACATGAACCTAGGCCTGGCCTTCACCAAGAACCGAATGACTATACCAACA
H W E H E L G L A P T K N R M N Y T N K
PQE60
961 AATTCTGTGATCCAGAGTCGGGAGACTACTTCATTTACTCCAGGTCACATTCCGTG
F L L I P E S G D Y F I Y S Q V T F R G
1021 GGATGACCTCTGAGTGCAGTGAAATCAGACAAGCAGGCCGACCAACAAGCCAGACTCCA
M T S E C S E I R Q A G R P N K P D S I
1081 TCACTGTGGTCATCACCAAGTAACAGACAGCTACCCTGAGCCAACCCAGCTCCTCATGG
T V V I T K V T D S Y P E P T Q L L M G
1141 GGACCAAGTCTGTATGCGAAGTAGGTAGCAACTGGTTCCAGCCCATCTACCTCGGAGCCA
T K S V C E V G S N W F Q P I Y L G A M
1201 TGTTCCTTCAAGAAGGGGACAAGCTAATGGTGAACGTCAGTGACATCTCTTTGGTGG
F S L Q E G D K L M V N V S D I S L V D
1261 ATTACACAAAAGAAGATAAAACCTTCTTTGGAGCCTTCTTACTATASGAGGAGAGCAAAT
Y T K E D K T F F G A F L L *

538 d1
448 d2
454 d3

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